SYNTHESIS OF THE HETEROAROTINOID ETHYL (*E*)-4-[2-(3,4-DIHYDRO-4,4-DIMETHYL-2*H*-1-BENZOPYRAN-6-YL)-1-PROPENYL]BENZOATE-*9,10,11,20-*$^{14}C_4$

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SUMMARY

A synthesis of ethyl (*E*)-4-[2-(3,4-dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propenyl]benzoate-*9,10,11,20-*$^{14}C4$ (**1**) is described via a multistep procedure similar to that used to obtained the unlabelled compound **2**. The latter has shown good activity in several assays compared to the standard *trans*-retinoic acid (**3**). Treatment of methyl 3-phenoxypropionate (**4**) with methylmagnesium iodide (obtained from $H_3{}^{14}C$-I) yielded the labelled tertiary alcohol **5**. Cyclization of the alcohol **5** occurred in the presence of $AlCl_3$ in nitromethane to give 4,4-dimethylchroman (**6**). Acetylation of **6** with $H_3C^{14}C(O)Cl$ produced ketone **7** labelled at three carbons. Reduction of the carbonyl group in **7** was effected with $LiAlH_4$ and gave alcohol **8**. Phosphorylation with triphenylphosphine hydrobromide in methanol led to the corresponding phosphonium salt **9**. Addition of *n*-butyllithium to **9** in ether at -78°C generated the expected Wittig reagent *in situ*, and to this was added labelled ethyl 4-formylbenzoate [$C_2H_5O_2{}^{14}C$-C_6H_4-CHO] (**10**). Workup, followed by chromatography of the oily product, afforded a solid. Recrystallization (abs. ethanol) gave **1** which was identical to **2** in terms of spectral data and melting point. The specific activity was determined to be 0.15 μCi/mg.

Key Words: Ethyl (*E*)-4-[2-(3,4-dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propenyl]benzoate-*9,10,11,20-*$^{14}C_4$, heteroarotinoid, Grignard addition, cyclialkylation, reduction, phosphorylation, Wittig condensation, anticancer agent

INTRODUCTION

A few heteroarotinoids have been synthesized and have exhibited good pharmacological activity in such assays as the tracheal organ culture (1,2), the ornithine decarboxylase assay (1-3) and, to a limited degree, in the differentiation of HL-60 cells (3). In the ornithine decarboxylase (ODC) assay (1,2), ethyl (*E*)-4-[2-(3,4-dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propenyl]benzoate (**2**) exhibited excellent activity compared to the standard *trans*-retinoic acid (**3**) (2). Both NMR (1,2) and UV (4) analyses

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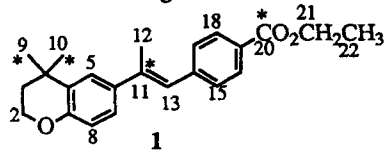
suggested that both rings in **2** were twisted and only partially conjugated with the internal



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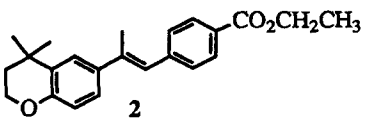

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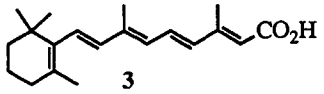

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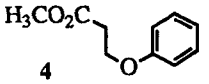

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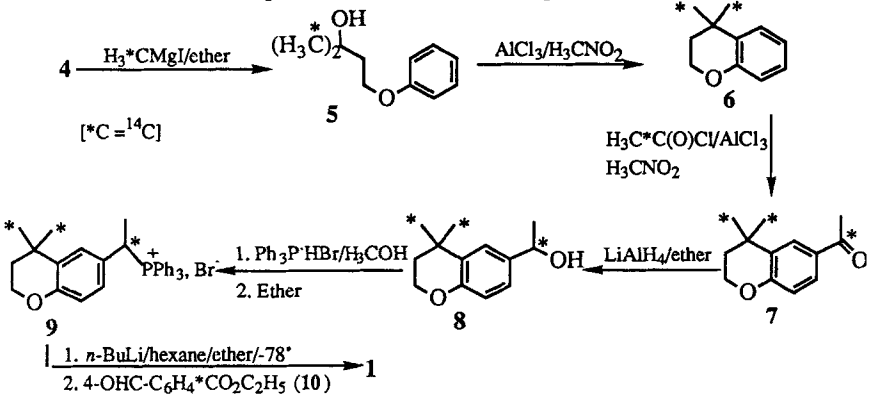
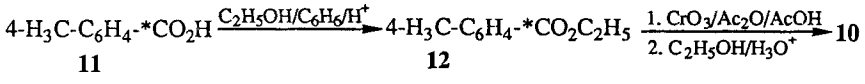
double bond in solution. The synthesis of labelled **1** is reported herein with the objective to determine the pharmacokinetics and the metabolites generated in animals. An assessment of the metabolism of *trans*-retinoic acid (**3**), as well as that of a few natural and synthetic retinoids (5,6), prompted us to introduce the ^{14}C label in positions 9-11 and 20. The scheme for the overall process is shown below and parallels, to some degree, that



described for the unlabelled **2** (1). Ester **4** (1) and the labelled compounds (ICN and Sigma) are available. Since intermediate **10** is not commercial, it was synthesized from commerical **11** as shown below. All properties of **10-12** were in accord with the known unlabelled compounds.



EXPERIMENTAL

2-Methyl-4-phenoxy-2-butanol-*1,2'*-$^{14}C_2$ (**5**). To 0.0698 g (2.91×10^{-3} mol) of Mg turnings in 0.5 mL of dry ether was added methyl iodide (^{14}C, 2.5 mg, 1 mCi, 56.6 mCi/mmol sp. act., ICN) along with 10 mL of dry ether followed by the addition of 0.531 g (3.74×10^{-3} mol) of H_3CI. After stirring at room temperature for 30 min, the reaction mixture was treated with 0.150 g (8.32×10^{-4} mol) of methyl 3-phenoxypropionate (**4**) (1)

in 5 mL of dry ether. After 24 h of stirring, the grey reaction mixture was poured onto 20 g of crushed ice. Ether (20 mL) was added followed by slow addition of saturated NH_4Cl solution (~40 mL). When the pH of the mixture was made neutral to litmus, the organic layer was separated. Extraction (ether, 10 x 10 mL) of the aqueous layer followed. The combined organic layer and extracts were washed with water (3 x 10 mL), saturated $NaHCO_3$ (3 x 10 mL), and saturated NaCl (20 mL). After drying (Na_2SO_4) and filtration, the solution was concentrated to give 0.190 g (9.99 x 10^{-4} mol) of a light yellow colored oil. This slightly impure **5** (1) was used in the next step without further purification since all spectral properties were identical to those of the unlabelled material.

4,4-Dimethylchroman-*9,10*-$^{14}C_2$ (6). To a suspension of 0.163 g (1.23 x 10^{-3} mol) of $AlCl_3$ in 2 mL of freshly distilled H_3CNO_2 was added 0.170 g (9.43 x 10^{-4} mol) of slightly impure **5** in 5 mL of freshly distilled H_3CNO_2. After stirring for 24 h at room temperature, the dark red reaction mixture was cooled in an ice-water bath for a few minutes. Treatment with HCl (6 M, ~10 mL) to a pH acidic to litmus produced two layers. Stirring of the mixture continued for 10 min and then ether (10 mL) was added.. Extracts (ether, 10 x 10 mL) of the aqueous layer, combined with the original organic layer, were washed with water (5 x 10 mL), saturated $NaHCO_3$ (4 x 10 mL), and saturated NaCl (15 mL). After drying (Na_2SO_4), the solution was concentrated to a light brown-colored oil (0.160 g, 9.86 x 10^{-4} mol) which was slightly impure **6** via spectral analysis (1). This material was used without further purification in the synthesis of **7**.

4,4-Dimethylchrom-6-yl Methyl Ketone-*9,10,11*-$^{14}C_3$ (7). To 1.57 mg (2 x10^{-5} mol, 1 mCi, 50 mCi/mmol sp. act., ICN) of $H_3C^{14}C(O)Cl$ in 5 mL of freshly distilled H_3CNO_2 was added 0.093 g (1.18 x 10^{-3} mol) of freshly distilled acetyl chloride. To this solution was added 0.160 g (9.86 x 10^{-4} mol) of the above 4,4-dimethylchroman (**6**) in 10 mL of freshly distilled H_3CNO_2. Slow addition of $AlCl_3$ (0.197 g, 1.48 x 10^{-3} mol) to the stirred solution followed. After stirring at room temperature for 10 h, the solution was cooled (ice-water bath). Cautious addition of 10 mL of 6 M HCl followed until the soltution was acidic to litmus. The combined organic layer and the ether extracts (10 x 10 mL) of the aqueous layer were washed with water (5 x 10 mL), saturated $NaHCO_3$ (3 x 10 mL), and saturated NaCl (15 mL). After this solution was dried (Na_2SO_4), filtered, and concentrated, a light brown oil (0.165 g, 8.08 x 10^{-4} mol) remained. This slightly impure **7** showed the characteristic IR band for the C=O group at 1650 cm^{-1} along with

other spectral properties which confirmed the known structure (1). The sample of **7** was used without further purification.

α,4,4–Trimethylchroman-6-methanol-*9,10,11*-$^{14}C_3$ (**8**). To a stirred suspension of 0.042 g (1.10 x 10^{-3} mol) of $LiAlH_4$ in 2 mL of dry ether was added ketone **7** from above in 5 mL of dry ether. The mixture was held at reflux for 24 h with about 15 mL of ether being added to maintain volume. After the mixture was allowed to cool to room temperature, it was chilled (ice-water). Addition of 6 M HCl was performed until the mixture was acidic to litmus. The combined organic layer and ether (10 x 10 mL) extracts were washed with water (5 x 10 mL), saturated $NaHCO_3$ (3 x 10 mL), and saturated NaCl (20 mL) solution. After the solution was dried (Na_2SO_4), it was was filtered and concentrated to a thick brown oil which was slightly impure alcohol **8** as indicated by spectral analysis (1). The compound was used directly in the next step.

[1-(4,4-Dimethylchroman-6-yl)ethyl]triphenylphosphonium Bromide-*9,10,11*-$^{14}C_3$ (**9**). A mixture of 0.120 g (5.82 x 10^{-4} mol) of alcohol **8** and 0.230 g (6.69 x 10^{-4} mol) of triphenylphosphine hydrobromide (1) was stirred at room temperature for 24 h. Evaporation of the methanol left a light brown oil which was triturated repeatedly with dry ether (~25 mL) until a solid formed. Filtration of the mixture provided a fair yield of **9** (0.210 g, 3.95 x 10^{-4} mol, 68%). The IR spectrum of this salt **9** was essentially identical to that reported for the unlabelled compound (1), and thus the former was used immediately in the final condensation.

Ethyl (*E*)-4[2-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propenyl]benzoate-*9,10,11,20*-$^{14}C_4$ (**1**). A solution of *n*-butyllithium (1.5 mL of 1.0 molar in hexanes-Aldrich) was added to 0.200 g of phosphonium salt **9** (approximately 0.150 g of labelled material and 0.050 g of cold material) in dry ether (20 mL). over a period of 2 min. The resulting dark red mixture was cooled to -78°C (dry ice-acetone bath), and then 0.085 g (0.477 mmol) of ethyl 4-formylbenzoate [^{14}C(O)OEt] (**10**) in ether (5 mL) was added. A cream-colored mixture formed, and this was stirred at -78°C for 10 min and then allowed to rise to room temperature with stirring over 24 h. A suspension formed and was filtered. The filtrate and one ether wash (25 mL) of the filtered solid were combined and evaporated to a yellow oil. Chromatography of this oil was effected on a Chromatotron (4 mm plate, silica gel 60 PF_{254} containing gypsum, model 7924T, Harrison Research Inc., 840 Moana Court, palo Alto, CA 94306) using 200 mL of 9:1 hexanes:ethyl acetate

as eluent. Two bands with the highest R_f values were collected, and the solvent was evaporated to give colorless oils. The oils appeared to be identical and were combined and dissolved in a minimum amount of hot absolute alcohol (~5 mL). The solution stood at room temperature for 1 h and then was refrigerated for 2 days. A white solid was deposited and this was filtered to obtain 13.2 mg (10%) of **1**. The specific activity was determined on a 10μ aliquot (prepared by dissolving 7.3 mg of **1** in 4 mL of HPLC grade methanol) via the use of a TRI-CARB liquid scintillation analyzer (model 1900-CA,Packard Instrument Company, Downers Grove, IL). The specific activity of **1** was 0.15 μCi/mg or 4.28×10^{-5} μCi/mmol. An average count of 6043.8 DPM was obtained. A mixture melting point determination of this material with an authentic sample of cold **2** did not show a depression (mp 72-73°C).

Ethyl *p*-Toluate-^{14}C=O (**12**). To a mixture of 0.015 g (1.10×10^{-4} mol, 0.5 mCi, 4.5 mCi/mmol sp. act., Sigma) of *p*-toluic acid-^{14}C=O (**11**) and 0.290 g (2.13×10^{-3} mol) of *p*-toluic acid was added 10 mL of absolute alcohol and 20 mL of dry benzene along with 1 mL of conc. sulfuric acid. After boiling the solution for 24 h, the near theoretical amount of water was collected via a Dean-Stark trap. Water (~20 mL) was added to the solution which had been allowed to cool to room temperature. The combined organic layer and ether extracts (10 x 10 mL) were washed with water (3 x 10 mL), saturated $NaHCO_3$ (3 x 10 mL), and brine (20 mL). After the solution was dried (Na_2SO_4), filtered, and concentrated, the light yellow liquid **12** remained and was used immediately in the next step. The weight of the oil was 0.380 g (qt).

Ethyl 4-Formylbenzoate-^{14}C(O)OEt (**10**). A solution of the oil (0.380 g, 2.31×10^{-3} mol) **12** in 5 mL of freshly distilled acetic anhydride and 5 mL of glacial acetic acid was cooled to 0°C (ice-water bath). Concentrated H_2SO_4 (0.2 mL) was added along with 0.693 g of CrO_3 in three equal portions over a period of 30 min. Care was taken to maintain the temperature below 5°C during the addition. When the addition was complete, a dark green reaction mixture remained which was stirred for 1 h at 0°C. Decompostion was effected by slowly pouring the mixture onto crushed ice (25 g) and then adding very slowly 50 mL of cold water. A green-colored solution formed, and this was extracted with $HCCl_3$ (10 x 10 mL). The extracts were washed with water (3 x 10 mL), 5% Na_2CO_3 (3 x 10 mL), and brine (20 mL). The dried (Na_2SO_4) solution was filtered and concentrated to give 0.385 g of a yellow oil. Water (10 mL), 95% ethanol (10 mL), and

conc. H_2SO_4 (0.5 mL) were added, and the resulting solution was held at reflux for 1 h. After cooling to room temperature, the solution was diluted with water (10 mL) and then extracted with $HCCl_3$ (10 x 10 mL). The extracts were washed with water (3 x 15 mL), 10% $NaHCO_3$ (3 x 10 mL), and brine (20 mL). When dried (Na_2SO_4), the solution was filtered and concentrated to give 0.090 g (36.8%) of **10** which was used in the Wittig reaction with the anion from salt **9** to yield **1**.

RESULTS AND DISCUSSION

The introduction of labels at C(9,10) occurs in the initial Grignard reaction to give alcohol **5** while the label at C(11) occurs in **7** obtained by the acetylation of **6**. The conditions reported herein for the syntheses to **7** have been optimized from those recorded (1). Direct introduction of the label at C(20) arises in the final reaction involving the Wittig reagent of salt **9** in a condensation with aldehyde **10**. The yields are comparable to those previously stated (1). Since all intermediates were confirmed by spectral analysis and by comparison of physical properties with those of authentic samples, the ^{14}C isotope is intact.

The modest activity found for **1** implies that the initial radioactivity was either less than stated by the manufacturer or the label was lost to some degree during the reactions. The material is currently undergoing metabolic studies in animals.

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